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Antioxidant effects after coffee enema or oral coffee consumption in healthy Thai male volunteers

S Teekachunhatean[1,2], N Tosri[3], C Sangdee[1], R Wongpoomchai[4], W Ruangyuttikarn[5], C Puaninta[6] and S Srichairatanakool[4]

Abstract

We designed an open-label, randomized two-phase crossover study to investigate the antioxidant effects after single and multiple doses of a coffee enema versus coffee consumed orally. Eleven healthy subjects were randomly assigned to either receive a coffee enema (3 times weekly for 6 visits) or consume ready-to-drink coffee (2 times daily for 11 days). After a washout period, subjects were switched to receive the alternate coffee procedure. Blood samples were collected at specific time points for the determination of serum levels of glutathione (GSH), malondialdehyde (MDA) and trolox equivalent antioxidant capacity (TEAC). The findings showed that either single or multiple administrations of the coffee enema or orally consumed coffee doses seemed not to produce any beneficial effects to enhance serum GSH levels or to decrease serum MDA levels over the study period of 12 days. In contrast, mean serum TEAC levels at day 12 after the coffee enema and at days 6 and 12 after oral coffee consumption were significantly reduced from their corresponding baseline values. Thus, no beneficial effects with respect to an enhancement of serum GSH and TEAC levels or a decrease in serum MDA concentrations were demonstrated after coffee enema or orally consumed ready-to-drink coffee.

Keywords

coffee, malondialdehyde, glutathione, trolox equivalent antioxidant capacity

Introduction

Since the 19th century, the large intestine has been described as a sewage system where toxins accumulate and are absorbed, leading to the theory of 'autointoxication.'[1] Autointoxication is an ancient theory based on the belief that intestinal waste products can poison the body and might be a major contributor to many life-threatening diseases.[2,3] Laxatives and enemas therefore have been routinely recommended by some physicians to detoxify the body through the removal of accumulated waste from the colon.[4] The coffee enema, an alternative medical procedure performed by infusion of the coffee solution into the rectum through the anus, has been one of the oldest medical procedures and is in use today for 'detoxification' since Gerson introduced them in cancer therapy in the 1930s.[5,6] Some experts in the field of complementary and alternative medicine believe that caffeine from the coffee enema causes dialysis of toxic products from blood across the colonic walls as well as to cause dilation of the bile ducts, which facilitates excretion of toxic products by the liver.[7]

[1] Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Center of Thai Traditional and Complementary Medicine, Chiang Mai University, Chiang Mai, Thailand
[3] Phisaleeh Hospital, Ministry of Public Health, Nakhonsawan, Thailand
[4] Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[5] Department of Forensic Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[6] Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Corresponding author:
Somdet Srichairatanakool, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
Email: ssrichai@med.cmu.ac.th

Thus, the coffee enema is claimed to have a very specific purpose in lowering serum toxins.[8]

It has also been shown that substances found in coffee, for instance kahweol and cafestol, are potent enhancers of glutathione *S*-transferase (GST), a major antioxidant enzyme that catalyses the binding of a vast variety of electrophiles in the blood stream to the sulfhydryl group of glutathione (GSH).[9] In mice, for example, coffee beans enhance this system by 600% in the liver and 700% in the bowel. Some researchers have shown that the average consumption of coffee in Italian drinkers increases the plasma concentration of GSH by 16%.[10] Additionally, melanoidins formed during the roasting of coffee beans exhibit strong antioxidant activity[11] and significantly inhibit lipid oxidation.[12] Phenolic compounds in coffee such as chlorogenic acid and caffeic acid have antioxidant activity in vitro,[13] whereas caffeine and its metabolites, theobromine and other xanthines appear to possess strong DNA-protective effects.[14] Thus, some practitioners propose that a coffee enema can eliminate toxins and exert antioxidant effects in the same or even superior manner to that of consumed coffee.[8] However, direct studies regarding the antioxidant effects of coffee enemas have been reported.

The widely claimed benefits of the coffee enema to detoxify the detrimental substances believed to cause cancers, allergy, asthma, urticaria, migraines and serious illnesses lead to its high popularity in some countries including Thailand, but as of yet have not provided adequate scientific support of its benefits and safety. Thus, the purpose of this study is to determine the antioxidant effects in serum after both single and multiple doses of coffee enema and orally consumed coffee doses.

Methods

Study design and subjects

The study was an open-label, randomized two-phase crossover study with at least a 10-day washout period. The study was approved by the Human Research Ethic Committee of the Faculty of Medicine, Chiang Mai University and complied with the Helsinki Declaration.

A total of 11 healthy men were enrolled in this study. Their body mass index (BMI) had to be within 18–25 kg/m^2. All had to be in good health on the basis of medical history and physical examination including normal blood pressure and heart rate. Routine blood testing including complete blood count as well as analyses of blood urea nitrogen, creatinine, aspartate aminotransferase and alanine aminotransferase levels were performed to exclude subjects with abnormal hematological diseases, or abnormal kidney or liver function. During the screening phase, subjects had to be capable of retaining a water enema for at least 5 min. Signed informed consent from each subject was obtained prior to the study. Subjects who could not avoid foods or drinks that contained caffeine within the previous 10 days and during the study period were excluded, as well as those with known histories of gastrointestinal diseases such as peptic ulcers, hemorrhoids, diverticulitis, ulcerative colitis, Crohn's disease, irritable bowel syndrome (IBS), recent bowel surgery and colorectal cancer. Other exclusion criteria were chronic renal, liver, neurological, pulmonary or cardiovascular diseases, recent cigarette smoking (within the previous 3 months), history of substance abuse or addiction, use of any medications or vitamins within the previous month, and hypersensitivity to medications in the xanthine group such as theophylline or aminophylline. All subjects were instructed to restrict their consumption of fruit juice to 2 glasses or less per day (total 300 ml) and not to change their dietary habits throughout the study period.

Coffee enema procedure

The coffee solution used in the enema procedure was prepared according to commonly practiced procedures carried out in Thailand, by mixing 4 g of finely ground coffee beans with 100 ml of purified water. VS® coffee manufactured by V.S. Coffee, Thailand, was used for the coffee enema. The solution was boiled at 100°C for 3 min and then simmered at 60°C for approximately 15 min. Afterward the solution was filtered using a very fine sieve, adjusting the total volume to 500 ml and it was then allowed to cool to 37°C.

The coffee enema device used in this study was a disposable commercial set (Cleansing Enema set® made in Mexico, imported by Thanyaphu Co. Ltd, Thailand) consisting of a plastic nozzle connected by a tube to a plastic bag containing the coffee enema fluid. The nozzle was lubricated with 2 drops of organic olive oil and then inserted 2 in deep into the anus while the subject was lying down on his left side, with his legs curled into his abdomen. The bed height was 3 ft above the floor, whereas the enema bag was hung 5 ft above the floor. The coffee solution in the

Table 1. Schedule of the administration of coffee procedures and blood sample collection during both phases of the study

Coffee procedure		Study period (day)											
		1	2	3	4	5	6	7	8	9	10	11	12
Coffee enema	Morning	√		√		√		√		√		√	
Coffee consumption	Morning	√	√	√	√	√	√	√	√	√	√	√	
	Evening	√	√	√	√	√	√	√	√	√	√	√	
Blood collection		[a]					[b]						[b]

[a]Blood sample collections at baseline and at 10, 20, 30, 40 and 60 min and 1.5, 2, 4, 8 and 12 h after the first coffee enema or consumption of the coffee beverage.
[b]Blood sample collections in the morning before the administration of coffee enema or the coffee beverage.

enema bag was completely infused within 5–10 min. The subject was requested to retain the coffee enema fluid for 10 min. During this period, the subject was instructed to change his lying position to the right side for 3.5 min and then to switch back to the left side for 3.5 min and finally in the supine position for 3 min before defecation. The enema procedure was performed at the Clinical Pharmacology Unit, Department of Pharmacology, Faculty of Medicine, Chiang Mai University.

Oral coffee consumption procedure

The coffee used for oral consumption in this study was the commercially available ready-to-drink coffee beverage, Red Bull Coffee® manufactured by T.C. Pharmaceutical's industry Co., Ltd. The net volume of 1 serving was 180 ml. Each subject was instructed to consume the entire coffee serving within 1 min followed by 100 ml of water.

Caffeine content in coffee solutions and schedule of coffee procedures

Six servings of each coffee solution were measured for their caffeine contents. Quantification of caffeine content in the coffee enema solution using high-performance liquid chromatography method revealed a mean content of 107 $\pm$ 2 mg/500 ml for the coffee enema solution and 96 $\pm$ 1 mg/180 ml for the orally consumed coffee solution.

In the first phase of the study, subjects were randomly assigned to either receive the coffee enema (500 ml each time, in the morning of every other day, for 6 visits) or orally consumed coffee beverage (180 ml each time, 2 times daily, 30 min before breakfast and dinner, for 11 days). After a washout period of at least 10 days, subjects were switched to the second phase receiving the alternate coffee preparation. All subjects were instructed to restrict their consumption of fruit juice not to exceed 2 glasses/day (total 300 ml) and not to change their dietary habits throughout the study period. During both study phases, blood samples collected at different time points on the first visit were used to determine the acute antioxidant effects after initiation of each coffee procedure, whereas those collected on day 6 and after completion of each study phase (day 12) were used to determine antioxidant effects following multiple doses of each coffee procedure (Table 1).

Blood sample collection

On the first visit of both the coffee enema phase and the oral coffee consumption phase, subjects were instructed to fast overnight for at least 8 h prior to the visit. Venous blood samples were taken via heparinized intravenous catheter inserted into a forearm vein. Fifteen milliliters of venous blood samples were drawn from each subject prior to either the coffee enema or oral coffee consumption and again at 10, 20, 30, 40 and 60 min and at 1.5, 2, 4, 8 and 12 h after each procedure. After initiating each coffee procedure, subjects continued fasting, water and lunch were served at 2 and 6 h afterward, respectively. After the last blood sample collection at 12 h, only subjects in the oral coffee consumption phase received the evening dose of coffee. Then, all subjects continued their multiple doses of coffee regimen on the corresponding phase described above.

The blood samples taken following multiple doses of either coffee treatment were collected in the morning during the study period (day 6) and again after completion of either treatment phase (day 12). The antioxidant parameters included serum levels of GSH, malondialdehyde (MDA) and trolox equivalent antioxidant capacity (TEAC). Seven milliliters of these blood samples were collected into clot activator test

tubes. Serum samples were separated within 1 h of extraction by centrifugation at 2250 r/min for 30 min and were stored at −20°C until analysis.

Determination of serum GSH concentration

GSH levels were measured using the method described by Ellman[15] and deproteinization was achieved using 10% meta-phosphoric acid. The deproteinized supernatant was stabilized by the addition of 4 M triethanolamine reagent. The supernatant can be stored at −20°C for up to 6 months for assay. The enzymatic recycling method used glutathione reductase (GR) for the quantification of serum GSH concentrations. Duplicates of 25 µl of the supernatant or standard or blank solutions were reacted with 150 µl of a mixture consisting of 4 mM nicotinamide adenine dinucleotide phosphate, 10 mM sodium phosphate buffer containing 5 mM ethylenediaminetetraacetic acid pH 7.5 and 6 U/ml GR, at 37°C for 5 min. Then, 6 mM of 5-5-dithio-bis (2-nitrobenzoic acid) (Ellman's reagent) solution was added and the solution was incubated at room temperature for 30 min. The absorbance of the mixture was measured at 405 nm. The GSH concentration was obtained from a standard curve prepared using 4, 8, 16 and 32 µM of GSH in deionized water.

Determination of serum MDA concentration

The MDA assay was based on the reaction of MDA with thiobarbituric acid (TBA) to form thiobarbituric acid reactive substances (TBARS). MDA levels in serum were measured by modifying the procedure according to Smith et al.[16] Briefly, serum samples were prepared by adding 4 ml of 3 N sulfuric acid (H_2SO_4) to 200 µl of serum and the mixture was gently shaken. Then 500 µl of 10% phosphotungstic acid was added and the sample was mixed. After allowing to stand at room temperature for 5 min, the mixture was centrifuged at 3000 r/min for 15 min. The resulting pellets were mixed with 2.0 ml of 3 N H_2SO_4 and 300 µl of 10% phosphotungstic acid. The mixture was centrifuged at 3000 r/min for 15 min. After removing the supernatant, the sediment was suspended in 4.0 ml of deionized water. Two milliliters of 0.33% TBA was added to the standard MDA solution which was resuspended. After agitation in a vortex mixer, the reaction mixture was heated for 1 h in a boiling water bath. Five milliliters of *n*-butanol was added and the mixture was shaken vigorously after being cooled to room temperature and centrifuged at 3000 r/min for 15 min. The absorption of the pink chromogen present in the *n*-butanol layer was recorded at 530 nm in a double-beam spectrophotometer. A standard curve was constructed using 0.01, 0.02, 0.04 and 0.08 mM 1,1,3,3-tetramethoxypropane. TBARS concentration was determined from the standard curve and reported as MDA equivalent.

Determination of serum TEAC

Serum TEAC was determined according to 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation ($ABTS^{\bullet+}$) decolorization assay.[17,18] Briefly, 1.0 ml of working $ABTS^{\bullet+}$ solution was incubated with 10 µl of serum or phosphate buffered saline–buffered trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution at 30°C for 6 min, the absorbance was immediately measured at 734 nm against the reagent blank. The antioxidant capacity (TEAC) values of the serum samples were calculated and determined from the calibration curve. A calibration curve of the TEAC assay was prepared by plotting trolox concentrations of 0.25, 0.5, 0.75 and 1.0 mM (x axis) against their mean optical densities at 734 nm (y axis).

Data and statistical analysis

All statistical analysis was performed using the SPSS package for Windows. All data were compared by the two-sided test. Differences were considered statistically significant at $p < 0.05$. The mean serum levels of GSH, MDA and TEAC between the baseline and at any specified time points, including day 6 and day 12 were compared using one-way analysis of variance (ANOVA) with repeated measurement. In addition, the mean changes from the baseline of these parameters at day 6 and day 12 of both coffee procedures were compared using paired t test.

Results

Subjects

Eleven healthy Thai male volunteers participated in the study. Their mean values of age, weight, height and BMI were 21.09 ± 7.97 yr, 58.86 ± 9.58 kg, 1.68 ± 0.07 m and 20.80 ± 2.27 kg/m^2, respectively. All completed the study protocol.

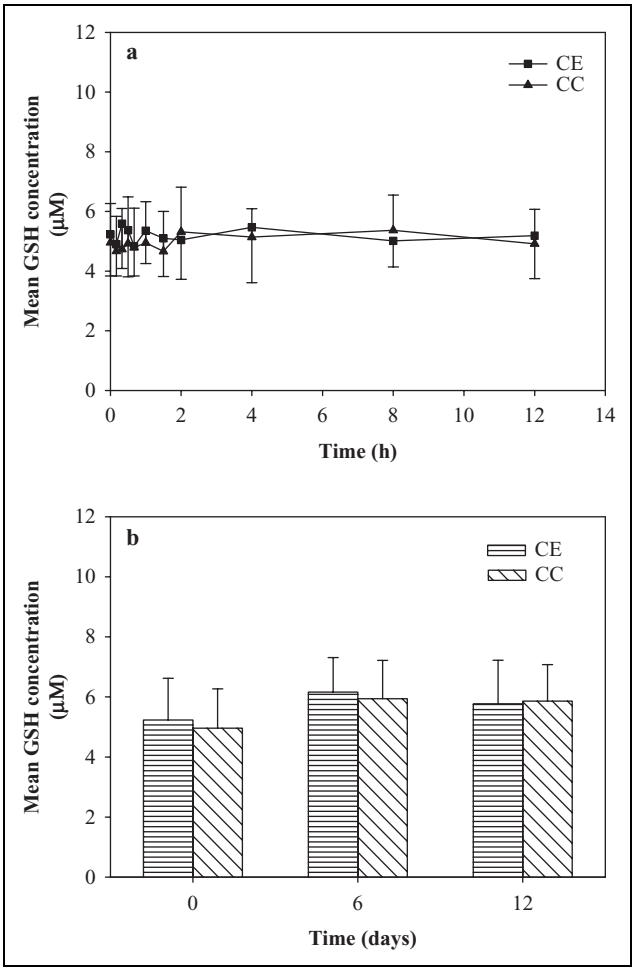

Figure 1. Effects of a single dose (a) and multiple doses (b) of the coffee enema (CE) or the coffee consumption (CC) on serum concentrations of glutathione (GSH).

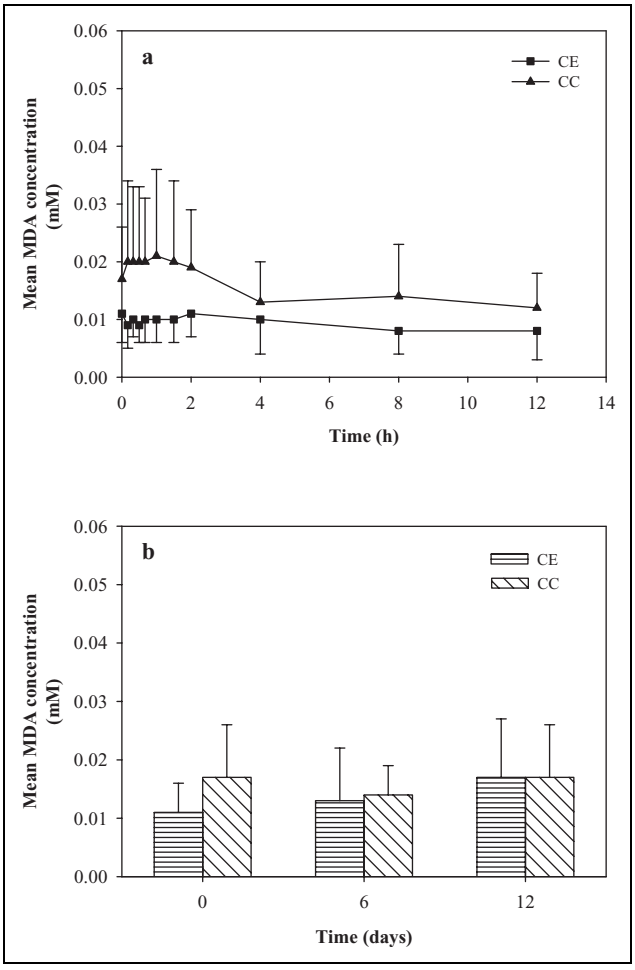

Figure 2. Effects of a single dose (a) and multiple doses (b) of the coffee enema (CE) or the coffee consumption (CC) on serum concentrations of malondialdehyde (MDA).

Effects of coffee procedures on serum levels of GSH, MDA and TEAC

As shown in Figures 1 and 2, the mean serum concentrations of GSH and MDA at baseline were not statistically different between the coffee enema and oral consumption phases. Single and multiple administrations of either the coffee enema or the oral coffee dose did not significantly alter serum concentrations of these parameters at any time points when compared with the baseline values.

The mean serum level of TEAC at baseline of the enema phase was coincidently higher than that of the oral phase ($p < 0.05$). A single dose of the coffee enema or a single dose of the orally consumed coffee did not significantly alter the serum levels of TEAC at any time points when compared to their own baseline values (Figure 3(a)). After multiple doses of the coffee enema, serum levels of TEAC significantly changed from the baseline value of 1.58 ± 0.09 to 1.48 ± 0.08 mM ($p < 0.005$) at day 12. Similarly, the average serum levels of TEAC after multiple doses of coffee consumption significantly changed from the baseline value of 1.51 ± 0.03 to 1.37 ± 0.17 mM ($p < 0.01$) and 1.39 ± 0.09 mM ($p < 0.05$) at days 6 and 12, respectively (Figure 3(b)).

The percentages of mean changes from the baseline of serum GSH, MDA and TEAC levels at days 6 and 12 after multiple oral doses of coffee consumption were not significantly different from those recorded from treatment with the coffee enema (data not shown).

Safety of coffee enema and oral coffee consumption

All subjects completed the study without any adverse events. The mean values of hemodynamic parameters

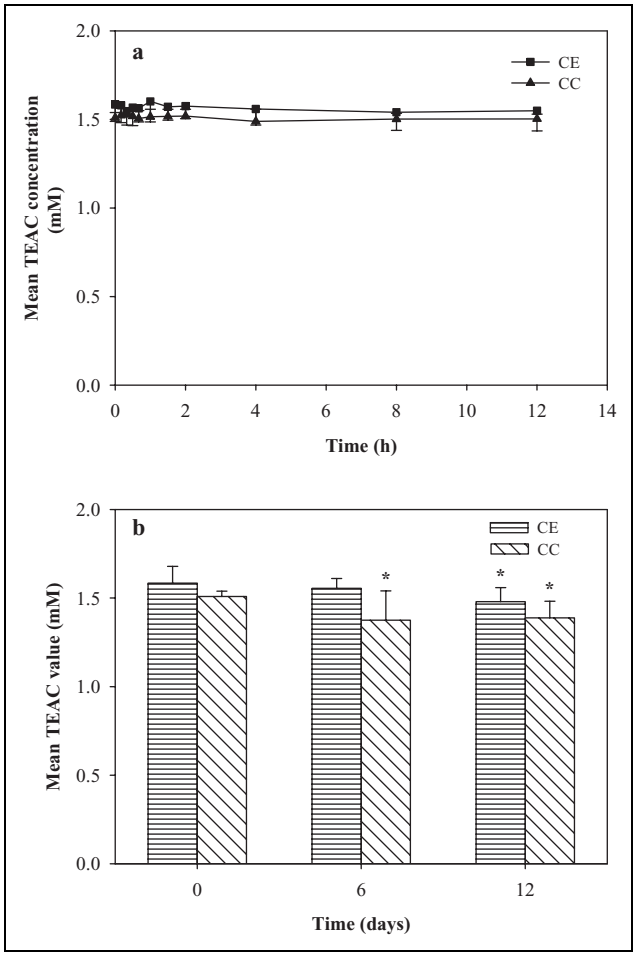

Figure 3. Effects of a single dose (a) and multiple doses (b) of the coffee enema (CE) or the coffee consumption (CC) on serum levels of trolox equivalent antioxidant capacity (TEAC). *Statistically significant difference from day 0 ($p < 0.05$, one-way analysis of variance (ANOVA) with repeated measurement).

(systolic blood pressure, diastolic blood pressure and heart rate) following multiple doses of each coffee procedure did not significantly alter from their own baseline values (data not shown). Additionally, the mean values of blood electrolytes following multiple doses of the coffee enema were 139 $\pm$ 2 mM for sodium, 3.99 $\pm$ 0.23 mM for potassium, 105 $\pm$ 2 mM for chloride and 26.4 $\pm$ 1.7 mM for bicarbonate, in comparison to the baseline values of 140 $\pm$ 2 mM for sodium, 4.25 $\pm$ 1.72 mM for potassium, 103 $\pm$ 2 mM for chloride and 22.1 $\pm$ 1.3 mM for bicarbonate. These differences in the mean values of blood electrolytes before and after the coffee enema were not clinically significant.

Discussion

Coffee is a rich source of caffeine and it also contains numerous substances, many of which are antioxidants, for example, melanoidins, polyphenolic compounds and diterpenoid alcohols.[9,11,13] Several lines of evidence suggest that coffee substances exert beneficial effects via the enhancement of endogenous antioxidant activities.

GSH serves as a nucleophilic cosubstrate to GST in the detoxification of xenobiotics and is an essential electron donor to glutathione peroxidases in the reduction of hydroperoxides.[19] In this study, average serum concentrations of GSH at the baseline were not statistically different between the coffee enema and oral consumption phases. These baseline GSH concentrations are comparable with the values of 5.5 $\pm$ 1.8 [20] and 4.1 $\pm$ 1.4 μM[10] in healthy volunteers that have been reported in other studies. However, since a single dose of the coffee enema or orally consumed coffee did not significantly alter serum concentrations of GSH at any time points, these data suggest that both forms of acute coffee procedures might not produce any beneficial effects in terms of the enhancement of serum GSH levels. Even though tissue concentrations of GSH were not measured in this study, there is evidence to support that the concentration of plasma GSH reflects the intrahepatic concentration.[21]

Although multiple oral doses of the coffee beverage in the present study showed a trend of increasing serum GSH concentrations (by about 25%) at days 6 and 12, these effects did not reach statistically significant levels. Consistently, Misik and colleagues have demonstrated that orally consumed paper-filtered coffee daily over 5 days insignificantly alters plasma GSH levels in Austrian subjects.[22] In contrast, consumption of unfiltered coffee 1 L/day for 14 days or 5 cups/day for 7 days have been reported to significantly increase plasma GSH by approximately 15–16% and by 8% in colorectal mucosa.[10,20] These discrepancies might be due to our smaller sample size yielding an inadequate power of test, as well as resulting from different types or lesser quantities of coffee beverage consumed in this study. It has been postulated that brewed unfiltered coffee contains much more antioxidant substances than ready-to-drink coffee used in our study as well as paper-filtered coffee,[23] possibly resulting in a larger content of antioxidant substances that were absorbed into the systemic circulation following oral administration, and hence a

higher total antioxidant status in the plasma. Additionally, the diverse influence of coffee consumption on enhancement of serum GSH levels might be partly due to great ethnic diversities among various populations, such as polymorphisms of the subunits of glutamate cysteine ligase, the enzyme involving in the rate-limiting step in the GSH synthesis.[24]

Similarly, subjects who received multiple doses of the coffee enema also increased their serum GSH levels by about 22% at day 6 and 16% at day 12, but again these changes did not attain significant levels. We postulate that the trend toward this enhancement might be the result of the mechanical cleansing of the large intestine from the enema procedure, thus preventing absorption of toxic waste products including free radicals into the body. The reduction in the bioavailability of secondary waste products in the evacuation of the colon might cause preservation of endogenous antioxidants and lead to the trend toward an increase in serum levels of GSH. To verify the statistical difference in the enhancement of serum GSH from coffee enema versus ready-to-drink coffee, coffee enema procedure using a higher coffee concentration, a larger volume, an increased frequency of administration or a longer retention period of the enema fluid should be further studied. Nonetheless, the bioavailability and pharmacokinetics of several antioxidant substances following coffee enema compared to coffee consumption also warrants further investigation.

At baseline, serum MDA concentrations of both the coffee enema and the oral coffee consumption phases were not significantly different and were in close proximity to the results of previous studies.[25,26] Similarly, the values of baseline serum TEAC in the present study were comparable to those reported previously in healthy volunteers.[27,28] Single and multiple doses of the coffee enema or the orally consumed coffee beverage did not significantly affect serum levels of MDA or TEAC at any time point, suggesting that both treatments might not produce any antioxidant advantage in terms of lowering serum MDA or enhancing TEAC levels. Nevertheless, Natella et al. have reported significant increases in the plasma total radical-trapping antioxidant parameters (TRAPs) at 2 h after a singular dose consumption of 200 ml of brewed coffee in 10 healthy nonsmoking and moderate coffee drinkers (2–4 cups/day).[29] In addition, Yukawa et al. have demonstrated that drinking unfiltered coffee, 24 g total per day, for 1 week by 11 healthy volunteers significantly decreased the serum levels of MDA.[30] This discrepancy in the total antioxidant status following coffee consumption between the present study and the previous studies might be due to the differences in the antioxidant parameters measured, as well as the types/amount of coffee beverage consumed, as mentioned above. In addition, ethnic-specific polymorphisms should also be taken into consideration.

Despite a statistically significant reduction in serum levels of TEAC following multiple doses of either coffee treatment (especially at day 12), these reductions might not correlate to a clinical significance because previous reports have shown that the serum TEAC levels in pathologic conditions should be much lower than the values reported here, such as 0.79 ± 0.68 mM in patients with hypothyroidism[27] and 0.004 ± 0.00007 mM in patients with cardiovascular diseases.[17] However, the mechanisms underlying the significant reductions in serum TEAC levels following multiple doses of both coffee treatments are still unknown, despite a trend toward an enhancement of serum GSH.

This study suggests that multiple doses of either form of coffee treatment used in this study should not produce any beneficial antioxidant effects regarding a decrease in serum MDA levels or an enhancement of serum GSH and TEAC levels. However, the antioxidant effects of orally consumed coffee and coffee enema also warrant a further investigation in a specific population who manifest abnormal baseline antioxidant levels, such as heavy smokers or patients with diabetes, acquired immune deficiency syndrome or cancer.

Although beneficial effects from coffee enemas have not been clearly demonstrated, the procedure in the routine practice is unlikely to produce any adverse effects on the cardiovascular system or an electrolyte balance. The coffee enema could therefore be employed according to personal preference, unless specific contraindications (i.e. hemorrhoids, gut obstruction, diverticulitis, ulcerative colitis, Crohn's disease, IBS, colostomy, recent bowel surgery and colorectal cancer) dictate otherwise.

In conclusion, a single dose or multiple doses of either the coffee enema or the ready-to-drink coffee for up to 12 days appears not to have any beneficial effects with respect to an enhancement of serum GSH and TEAC levels or a decrease in serum MDA concentrations in the present experimental setting. Nonetheless, multiple doses of a coffee enema and the coffee beverage did not adversely affect either the homodynamic parameters or the electrolyte balance.

The major limitations of this study were the small sample size as well as the larger variations (indicated by high standard deviations) of serum levels of GSH and MDA measured at certain time points that might have contributed to some of the insignificant differences when they were compared to the corresponding baseline values. The insignificant alterations in serum levels of GSH and MDA found in this study might not fully reflect the fact that both coffee procedures were incapable of exhibiting antioxidant activity. Plasma levels of TRAP, the ferric reducing-antioxidant power, antioxidant enzymes (e.g. superoxide dismutase, catalase and glutathione peroxidase), phenolic acids (e.g. caffeic acid, ferulic acid and *p*-coumaric acid) as well as uric acid are potentially valuable biomarkers that reflect plasma antioxidant capacity in these subjects and therefore should warrant further investigation.

Authors' Note

ST initiated the research question, supervised data collection and analysis and drafted the manuscript. NT performed the quantification of the antioxidant parameters and statistical analysis. CS participated in the design of the study and edited the manuscript. SS, RW, WR, CP supervised the quantification of the edited antioxidant parameters. All authors read and approved the final manuscript.

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Declaration of Conflict of Interest

The authors declared no conflicts of interest.

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